

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT



(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 21134 PC 1	<div style="display: flex; justify-content: space-between;"> <div>FOR FURTHER ACTION</div> <div>See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)</div> </div>	
International application No. PCT/DK99/00382	International filing date (<i>day/month/year</i>) 02/07/1999	Priority date (<i>day/month/year</i>) 03/07/1998
International Patent Classification (IPC) or national classification and IPC C12N1/20		
Applicant CHR. HANSEN A/S et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
- ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).
- These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand 17/12/1999	Date of completion of this report 19.09.00
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**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/DK99/00382

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-25 as originally filed

Claims, No.:

1-27 as received on 13/07/2000 with letter of 10/07/2000

Drawings, sheets:

1/12-12/12 as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/DK99/00382

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims 1-27
	No: Claims
Inventive step (IS)	Yes: Claims 1-27
	No: Claims
Industrial applicability (IA)	Yes: Claims 1-27
	No: Claims

2. Citations and explanations

see separate sheet

- 1). The present application relates to a method of preventing bacteriophage infection of bacterial cultures due to a mutation which renders the bacteria incapable of DNA replication, RNA transcription or protein synthesis, but which retain their capability of being metabolically active. The application further relates to the so defined bacteria and their use in the production of edible products.
- 2). Novelty, Inventive Step and Industrial Applicability (Box V)

2.1). Prior Art

D1: RICHARDSON G H ET AL: 'Proteinase negative variants of Streptococcus cremoris for cheese starters' JOURNAL OF DAIRY SCIENCE, vol. 66, 1983, pages 2278-2286, XP002082743 cited in the application

D2: NILSSON D & LAURIDSEN A A: 'Isolation of purine auxotrophic mutants of Lactococcus lactis and characterization of the gene hpt encoding hypoxanthine guanine phosphoribosyltransferase.' MOLECULAR AND GENERAL GENETICS, vol. 235, 1992, pages 359-364, XP002032525 cited in the application

D1 discloses proteinase negative variants of Streptococcus cremoris which are able to acidify milk. However, these variants are still growing, although at a lower rate than the proteinase positive strain, as stated on page 2278 "Generation times of proteinase negative cells were 3.5 times as long in cheese milk as those of proteinase positive, suggesting a probable reduction of bacteriophage and antibiotic problems."

D2 discloses the purine auxotrophic mutant DN105 of L. lactis (paragraph spanning page 360, right col.-page 361, left col.). D2 does not refer to the use of said mutant in food production.

2.2). Novelty

Growth, as in the case of D1, requires DNA replication, RNA transcription and protein synthesis, therefore variants of D1 do not appear to fall under the requirements set out in claim 1, 2, 3, 8-11 and 26. Also the subject matter of

claims 4-7, 12-25 and 27 appears to be novel with regard to D1.

The strains disclosed in D2 were not used in the methods of claims 1-17 and 24-27. Also the subject matter of claims 20-23 appears to be novel with regard to D2. The mutants DN101-104 which are also disclosed in D2 are disclaimed in claim 18 and 19, therefore said claims can be regarded as novel (Art. 33 (2) PCT).

2.3). Inventive Step

The problem underlying the present application is that bacterial cultures, used for example for the fermentation of milk, are often subject to bacteriophage infection. This problem is solved by the provision of bacteria that are purine or pyrimidine mutants but still capable of metabolizing substrate material.

It is common knowledge that DNA replication, RNA transcription and protein synthesis is necessary for bacteriophage development.

However, with regard to the description page 17, lines 11-13, it was apparently not known that purine auxotrophic mutants of *L.lactis* not growing in milk would be capable of acidifying such a substrate material.

Therefore the use of a purine auxotrophic mutant of *L.lactis* in the method of claims 1-4, 6-11 and 27 involves an inventive step (overcoming of a prejudice).

The use of a pyrimidine (Pyr⁻) mutant is not obviously derivable from the prior art. Therefore claims 5 and 20-27 are inventive.

In the method of claims 12-14 the strain not being susceptible to attack by bacteriophages due to not being capable of DNA replication, RNA transcription or protein synthesis is, additionally, genetically modified such that it has an enhanced metabolic activity relative to its parent strain. Although not reduced into practice in the present application, it seems likely that the skilled person would be able to obtain such a strain with regard to the teaching of example 2 (page 18 of the present application) in combination with page 11 and the teaching of WO 98/10089.

In the method of claims 15 and 16 the feature "not being capable of DNA replication, RNA transcription or protein synthesis" should be due to a conditional mutation. Although not reduced into practice in the present application, it seems nevertheless likely that the skilled person would be able to obtain such a strain

using his or her general knowledge of methods to use to provide such a conditional mutant.

In the method of claims 17 a strain which is not capable of DNA replication is unable to carry out mitosis, but it would still be able of growing in size, since, under this condition synthesis of new enzymes is not affected.

Thus also the methods of claims 12-17 can be considered to involve an inventive step.

2.4). Industrial Applicability

The subject matter of claims 1-27 appears to be industrial applicable.